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Characterization of Fecal Extended-Spectrum-β-Lactamase-Producing *Escherichia coli* in a Remote Community during a Long Time Period

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Carriage of extended-spectrum beta-lactamase-producing enterobacteria (ESBL-E) has increased in community settings. Little is known about their long-term evolution. French Guiana Amerindians living in a very remote village, already sampled in 2001 and 2006 for ESBL-E fecal carriage, were screened again in October 2010. Sociodemographic data and antibiotic intake data were collected during the previous year. ESBL-E strains collected in 2010 and their plasmid contents were typed. The results were compared to those of the previous campaigns. The prevalence of ESBL-E carriage in 2010 was 5.3%, whereas it was 8.0% and 3.2% in 2006 and 2001, respectively. As previously determined, no individual factor was associated with carriage, including personal antibiotic exposure. However, overall antibiotic use had decreased to a 0.67 treatments/subject/year in 2010 versus 1.09 in 2006 ($P < 0.001$), which supports the idea that population exposure to antibiotics impacts on ESBL-E community carriage rates. A wide diversity of ESBL *Escherichia coli* strains belonging to the A_0, A_1, B1, and D_2 phylogroups and producing the CTX-M-1, CTX-M-2, and CTX-M-8 enzymes were isolated. Despite the overall genetic diversity of the strains evaluated by repetitive extragenic palindromic PCR (rep-PCR) and multilocus sequence typing, two CTX-M-1-producing clones were found to have spread. In contrast, similar ESBL-bearing I1/Iγ plasmids were present in various strains both within and between carriers, suggesting high rates of plasmid transfer. Our results suggest that overall antibiotic exposure affects ESBL-E fecal carriage in the community. ESBL-E spread may be the result of both strain dissemination and the transfer of plasmids in intestinal microbiota.

Infections caused by extended-spectrum beta-lactamase-producing enterobacteria (ESBL-E) are a major public health burden worldwide (1). Although ESBL-E infections for a long time were limited to hospitalized patients, this changed at the turn of the century, when a gradual increase was observed in community cases, together with a shift in the types of enzymes produced (from TEM/SHV mutants to CTX-M types). Intestinal colonization is far more frequent than infections, but it is one of the major determinants of infections (2). Antibiotic exposure (3) and travel in countries with high ESBL-E rates (4) have been reported to influence carriage. Unlike the plasmidic $bla_{\text{TEM-SHV}}$ mutants, $bla_{\text{CTX-M}}$ genes are wild variants that were only recently transferred from the chromosomes of environmental bacteria (1). In humans, $bla_{\text{CTX-M}}$ genes have apparently ended up on plasmids that are highly adapted to transfer and dissemination among *Escherichia coli* species (1). Also, some *E. coli* genetic and/or phenotypic backgrounds, such as sequence type 131 (ST131), might be highly adapted to humans and serve as privileged recipients for $bla_{\text{CTX-M}}$, which could further explain dissemination (1).

However, it is difficult to explore the dynamics of community dissemination of ESBL genes and the factors associated with their spread in open populations because the frequent movement of people from one place to another and changes associated with modern lifestyles prohibit the monitoring of stable cohorts of individuals over long periods of time. In addition, it is difficult to record precise antibiotic exposure of individuals in modern societies due to the multiplicity of sources, including various types of medical care, the food chain, living habits, and so forth. Since 2001, we have tried to overcome these difficulties by studying the Amerindian Wayampi community living in Trois-Sauts, a very remote village located in southeast French Guiana (South America) (5, 6). Between 2001 and 2006, both the carriage rate of ESBL *E. coli* and overall antibiotic exposure had increased in this community. This was associated with changes in the types of ESBL genes that mimicked those observed in the rest of the world (5, 6). Neither in 2001 nor in 2006 did we find any individual risk factor significantly associated with carriage, antibiotic intake included. Here, we report the results of a third sampling campaign performed in 2010, which aimed to analyze the long-term evolution of ESBL-E carriage in that community.

MATERIALS AND METHODS

Subjects. The study was performed in October 2010. The Trois-Sauts village then had a population of 597 inhabitants and around 10 civil servants, including school teachers and nurses. The lifestyle of the population was still largely traditional, without major changes compared to 2001 (5) and 2006 (6), although access by motor-driven boats and schooling of the oldest children outside the village at around 150 km to the north had

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increased. The only source of antibiotics was still the health post, where drugs continued to be delivered free of charge upon prescription by the resident nurses, who also kept a precise record of all distributions. As before, only visitors who benefit from permits which are rarely delivered by the regional authority were admitted in the village. We identified the 163 adult volunteers from the 2006 cohort and asked them to participate again. A small financial reward was given for their participation, as during our previous sampling campaign. Volunteers were included if they were healthy at physical examination and signed an informed consent form. Then, they were also expected to reply to a standard questionnaire and provide a freshly passed fecal sample. The study was approved by the Regional Ethics Committee (Comité de Protection des Personnes Sud-Ouest et Outre Mer III, 2010-A00682-37).

Bacteriological methods. The bacteriological techniques used were essentially the same as those used during the previous campaigns in order to ensure optimal comparative analyses. Fresh fecal samples were inoculated immediately onto Drigalski agar slants in screw-cup tubes and sent to mainland France within 2 weeks, at room temperature. There, the whole culture from each tube was suspended in 1.5 ml of brain heart infusion broth with 10% glycerol and stored at −80°C. Upon harvesting, 100-μl aliquots of each broth were cultured on agar plates selective for extended-spectrum cephalosporin-resistant strains containing 1.5 mg/liter cefotaxime and 2 mg/liter ceftazidime (ESBL; AES Chemunex, Bruz, France). All *Enterobacteriaceae* with different morphotypes that grew on these plates were identified at the species level using a Maldi Biotyper system (Bruker Daltonics, Bremen, Germany). Their antimicrobial susceptibility was determined using the disk diffusion method, as previously described (http://www.sfm-microbiologie.org/). Class A ESBLs were detected using a double-disk synergy test (7). The phylogenetic relationships of *E. coli* isolates were determined using the triplex PCR method (8) and multilocus sequence typing (MLST) (9), using 40 strains representing species diversity as references (10). Isolates of the present sampling were compared with each other and also with isolates from 2006 from the same volunteers by repetitive extragenic palindromic PCR (rep-PCR), using a DiversiLab strain typing system (bioMérieux, Marcy l'Etoile, France). The presence of extraintestinal (*hly*, *aer*, *papC*, *iroN*, *traT*, *ompT*, *fyuA*, *hra*, and *kpsE*) and intraintestinal (*stx1*, *stx2*, *ipaH*, heat-labile toxin [LT] and heat-stable toxin [ST] genes, *aatA*, *aaiC*, *afaD*, *eae*, and *bfpA*) virulence factor genes was assessed in isolates representative of each rep-PCR pattern, as described previously (10).

Resistance genes, including $bla_{\text{CTX-M}}$ (groups 1, 2, 8, 9, and 25), bla_{SHV}, and bla_{TEM}, were amplified with specific primers, as described previously (11). All amplification products were sequenced and submitted to the National Center for Biotechnology Information Library (http://blast.ncbi.nlm.nih.gov) for precise identification.

The transferability of ESBL genes was assessed by mating with *E. coli* J53rif (12). When mating was negative, transformation into *E. coli* TOP10 (Invitrogen, Saint-Aubin, France) was attempted by electroporation of whole-plasmid DNA, as described previously (6). Transformants were selected on Drigalski agar with 1 mg/liter cefotaxime, and their antimicrobial susceptibility patterns were assessed. Plasmid replicons from parental strains, transconjugants, and transformants were typed by PCR, as described previously (13). Finally, when plasmids from strains of different genetic backgrounds bore the same CTX-M allele and were either undistinguishable by replicon typing or shared one (or more) common replicon(s), plasmidic DNA was purified from *E. coli* transconjugants or transformants using the alkaline lysis procedure (Large-Construct kit; Qiagen, Courtaboeuf, France), as recommended by the manufacturer. Restriction fragment length polymorphisms of plasmid DNA were analyzed after cleavage with EcoRI and 0.8% agarose gel electrophoresis. The same procedure was also applied to the plasmids isolated from the same volunteers during the 2006 campaign, when relatedness was suspected according to the same criteria.

Epidemiological data. Demographic data (age, sex, marital status, and number of children), data on lifestyle (including travel frequency, duration, and destinations) and the environment (size and location of households, contact with animals, daily activities, and baby-sitting of children under 5 years old), and data on medical history (current pregnancy, chronic disease, previous hospitalizations and surgery, and oral and parenteral antibiotic intake during the year before sampling) were collected from each volunteer in a standardized manner using data recorded at the health post and interviews of local informers. Antibiotic treatments prescribed to all villagers were available for 92% of them (552/597) by extraction from the health records, which are stored in the health post. Familial status and the location of the household of each villager were also recorded.

TABLE 1 Comparison of Trois-Sauts villagers (2006 and 2010) and volunteers (2010)

Characteristic	Value for the parameter by study population and year			
	Villagers			2010 Volunteers (no. of subjects)
	2006 (no. of subjects)	2010 (no. of subjects)	P^a	
Population	525	597		151
Gender				
Female	278	318	0.96	85
Male	247	279		78
Age				
<18 yr	287	324	0.94	0
Adult	238	273		151
Location of the household (distance from the health post [m])			0.76	
Hamlet 1 (0)	320	355		94
Hamlet 2 (1,000)	129	152		38
Hamlet 3 (3,500)	36	36		15
Hamlet 4 (5,500)	40	54		16

[a] Comparisons of data from 2006 and 2010 were performed using Pearson's chi-square test ($\alpha = 0.05$).

Statistical methods. All data were analyzed blindly with respect to the identity of the villagers. However, each inhabitant was assigned a code number which allowed us to perform comparisons over time. We compared the epidemiological characteristics of ESBL-E carriers versus noncarriers by means of R software (version 2.13.0 [http://www.cran.r-project.org]). A univariate analysis of discrete variables was performed using a two-sided Pearson chi-square test and Fisher's exact tests. The Student *t* test and Wilcoxon's test were used to testing continuous variables. All tests performed were two sided, and the significance level was set at 5%. Due to the large number of explanatory variables tested, the results of the univariate analysis were adjusted using Holm's test for multiple testing. We evaluated for a 9-year period (from 2001 to 2010) the evolution of the ESBL-E carriage rate according to overall antibiotic exposure in the year preceding each campaign using a linear regression model and Pearson's correlation. We used McNemar's test to compare the ESBL-E carriage rates between 2006 and 2010.

RESULTS

Characteristics of the study population. The village population increased from 525 subjects in 2006 to 597 in 2010 without significant structural changes (Table 1). Of the 163 volunteers from the 2006 campaign, 152 were present in 2010, and 151 (99%) of them volunteered again (Table 1).

ESBL-E fecal carriage. The ESBL-E carriage rate dropped from

TABLE 2 Comparison of antibiotic exposure among volunteers and villagers in 2001, 2006, and 2010

Antibiotic name or class	Antimicrobial exposure by population group (no. of treatments/subject/yr)				
	Villagers			Volunteers	
	2001 (n = 388)[a]	2006 (n = 512)	2010 (n = 552)	2006 (n = 151)	2010 (n = 151)
Penicillin	0.19	0.58	0.43	0.40	0.31
Cephalosporin	0	0.04	0.03	0.03	0.01
Macrolide	0.09	0.13	0.09	0.15	0.12
Metronidazole	0.29	0.22	0.09	0.23	0.17
Ofloxacin	0	0.01	0.01	0.03	0.03
Other[b]	0.08	0.10	0.02	0.09	0.02
Total[c]	0.64	1.09	0.67	0.89	0.65

[a] n, number of subjects.
[b] Nitroxoline, doxycycline, and cotrimoxazole.
[c] For the total of each group, P values were <0.001 (chi-square test) for the villagers and 0.06 (Student t test for paired data) for the volunteers.

8.0% (13/163) in 2006 to 5.3% (8/151) in 2010. Three additional volunteers in 2010 (1.8%) carrying isolates were further identified as producing CMY-2 plasmid-borne cephalosporinase (see below) versus only one study volunteer in 2006.

Antibiotic exposure. Overall antibiotic exposure decreased in the village from 1.09 treatments/subject/year in 2006 to 0.67 treatments/subject/year in 2010 ($P < 0.001$), a figure close to that observed in 2001 (5) (Table 2). This decrease was observed for all antibiotic classes and was highest for penicillins and metronidazole, which were also the most frequently used antibiotics. This was also true for cohort volunteers ($P = 0.06$) (Table 2). Of note, carboxiiminopenicillins, cephalosporin, or carbapenems were not available, and exposure to fluoroquinolones was minimal (four courses of antibiotics during the year preceding the sampling campaign).

Risk factors for ESBL-E carriage. We found no significant differences in demographic data, lifestyle environment, or medical history between ESBL-E carriers and noncarriers after adjustment for multiple testing (Table 3). The overall level of antibiotic consumption in the village and the ESBL-E carriage rate are presented in Fig. 1. A trend toward a correlation was found using linear regression ($y = 8.675x - 1.396$; $R^2 = 0.87$). However, Pearson's correlation did not reach significance.

Genetic backgrounds. Among the eight ESBL-E carriers identified in 2010, four, three, and one were carrying, respectively, one, two, and three different isolates, all of which were *E. coli*. Thus, a total of 13 ESBL *E. coli* isolates were isolated (Table 4). As in 2006 in the same volunteers, most of the isolates (12/13) were from commensal A and B1 phylogroups (14). A single strain was from phylogroup D. Rep-PCR showed two patterns (M and N) that were shared each by three different isolates. Each pattern was present in three separate volunteers. Interestingly, isolates exhibiting patterns M and N coexisted in two of them (Table 4). These four volunteers (two carrying both isolates with the M and N patterns and two carrying only one isolate) lived in the same hamlet, and three of them were direct neighbors, although not family related. The other seven isolates were singletons, as confirmed by MLST (Fig. 2). Strikingly, rep-PCR patterns from 2010 isolates were all different from those of 2006 (see Fig. S1 in the supplemental material). Just as in 2006, the virulence gene content of ESBL-E isolates, which was analyzed in strains representative of the rep-PCR patterns, was low (Table 4). Of note, one strain was positive for *aatA* and *aaiC*, which allowed us to identify an enteroaggregative pathotype.

TABLE 3 Risk factor analysis for ESBL-E carriage in 151 volunteers (2010)

Parameter	Value for the parameter (no. [%])			
	Noncarriers (n = 143)	Carriers (n = 8)	Univariate OR (95% CI)[a]	P[b]
Sociodemographic data				
Gender				
Male	72 (50.3)	2 (25.0)	1.0	0.28
Female	71 (49.7)	6 (75.0)	3.0 (0.5–31.6)	
Age (yr)				
20 to 35	74.0 (51.7)	1 (12.5)	1.0	0.06
36 to 83	69.0 (48.3)	7 (87.5)	7.4 (0.9–342.3)	
Marital status				
Single	12 (8.4)	1 (12.5)	1.0	0.52
Couple	131 (91.6)	7 (87.5)	0.6 (0.1–31.3)	
No. of children				
0 to 4	84 (58.7)	2 (25.0)	1.0	0.08
5 to 15	59 (41.3)	6 (75.0)	4.2 (0.7–44.3)	
Household				
Location				
Hamlet 1	82 (57.3)	3 (37.5)	1.0	0.26
Hamlet 2	33 (23.1)	4 (50.0)	3.3 (0.5–23.6)	
Hamlet 3	15 (10.5)	0 (0.0)	0.0 (0.0–14.2)	
Hamlet 4	13 (9.1)	1 (12.5)	2.1 (0.0–28.3)	
No. of inhabitants/household				
2 to 6	75 (52.4)	4 (50.0)	1.0	1.00
7 to 12	68 (47.6)	4 (50.0)	1.1 (0.2–6.2)	
Animals in the household				
Presence	109 (76.2)	6 (75.0)	0.9 (0.2–9.9)	1.00
Dogs	54 (37.8)	1 (12.5)	0.2 (0.0–1.9)	0.26
Chickens	100 (69.9)	6 (75.0)	1.3 (0.2–13.5)	1.00
Lifestyle				
Drinking water[c]				
River	57 (41.3)	2 (25.0)	0.5 (0.0–2.8)	0.47
Cove	52 (37.7)	3 (37.5)	1.0 (0.1–5.3)	1.00
Tap water	87 (63.0)	6 (75.0)	1.8 (0.3–18.4)	0.71
Duty				
Hunting	70 (49.0)	2 (25.0)	0.3 (0.0–2.0)	0.28
Fishing	118 (82.5)	7 (87.5)	1.5 (0.2–69.5)	1.00
Manioc culture	130 (90.9)	7 (87.5)	0.7 (0.1–33.9)	0.55
Cooking	92 (64.3)	7 (87.5)	3.9 (0.5–178.1)	0.26
Cachiri preparing	74 (51.7)	6 (75.0)	2.8 (0.5–29.1)	0.28
Pirogue driver	83 (58.0)	4 (50.0)	0.7 (0.1–4.1)	0.72
Community worker	7 (4.9)	1 (12.5)	2.7 (0.1–27.2)	0.36
Babysitting children of ≤5 years of age	99 (69.2)	8 (100.0)	3.1 (0.4–143.0)	0.43
Travel outside Trois-Sauts during the past year[d]	124 (86.7)	6 (75.0)	0.5 (0.1–5.0)	0.31
Having a child at school outside Trois-Sauts[d]	36 (25.2)	3 (37.5)	1.8 (0.3–9.7)	0.43
Medical history during the past year[e]				
Pregnancy	5 (7.2)	0 (0.0)	0.0 (0.0–14.6)	1.00
Hospitalization	17 (12.0)	0 (0.0)	0.0 (0.0–4.7)	0.60
Surgery	5 (3.5)	0 (0.0)	0.0 (0.0–21.9)	1.00
Serious medical event	14 (9.9)	1 (12.5)	1.3 (0.0–11.4)	0.58
H1N1 vaccination	56 (39.4)	2 (25.0)	0.5 (0.0–3.0)	0.49
Antibiotic use	52 (36.6)	3 (37.5)	1.0 (0.2–5.6)	1.00
Antibiotic use among relatives[f]	126 (88.1)	6 (75.0)	0.4 (0.1–4.4)	0.26

[a] OR, odds ratio; CI, confidence interval. For the parameter age, multivariate analysis was performed using the adjusted P value for multiple testing by the Holm's method; the odds ratios (95% confidence interval) were 1 for the parameter 20 to 35 years of age and 7.5 (1.3 to 142.2) for the parameter 36 to 83 years of age.
[b] The univariate analysis was performed using the Pearson chi-square, Fisher's exact, Wilcoxon, or Welch test ($\alpha = 0.05$).
[c] Data from five subjects are missing.
[d] The most frequent locations for travel and schools were Camopi, Saint-Georges, and Cayenne, all in French Guiana.
[e] Data from one subject are missing.
[f] Relatives include members of the same family living in the same household. In the case of multiple life partners, second/third wives and children were included as relatives even if they lived in a different household. The number of relatives ranged from 1 to 11.

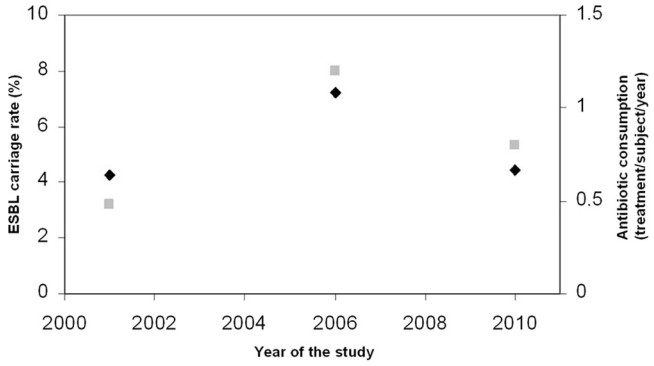

FIG 1 ESBL-E carriage rate in Wayampi volunteers (gray squares) and overall antibiotic exposure of the whole community (black diamonds) in 2001, 2006, and 2010. Linear regression ($y = 8.675x - 1.396$; $R^2 = 0.87$) and Pearson's correlation ($P = 0.24$) were used to evaluate the evolution.

Susceptibility pattern and identification of ESBL and cephalosporinases. ESBL-E strains were coresistant to sulfamethoxazole (77%, or 10/13), tetracycline (69%, or 9/13), trimethoprim (46%, or 6/13), nalidixic acid (23%, or 3/13), and kanamycin (8%, or 1/13). Three strains had no coresistance at all (Table 4).

All 2010 ESBL isolates were CTX-M types, including six (46%) CTX-M-1, five (38%) CTX-M-8, and two (15%) CTX-M-2 (Table 4). CMY-2 was identified in the three cases suspected of producing a transferable cephalosporinase. Transfer experiments showed that cotransfer of other resistant traits together with ESBL genes had occurred in only 4/9 cases and was limited to sulfamethoxazole, trimethoprim, and tetracycline (Table 4).

Plasmid characterization. Plasmids present in CTX-M-8 strains were positive for incompatibility group I1/Iγ in strains K, Q, R, and S isolated in 2010 but not typeable in strains A and P, isolated in 2006 and 2010, respectively. Restriction patterns of I1/Iγ plasmids from the four CTX-M-8 strains (K, Q, R, and S) were similar (β pattern) (Table 4). Of note, three of these strains (Q, R, and S) were cocarried by one volunteer, whereas the last strain (K) was found in another volunteer who was unrelated and lived in a remote hamlet. In the two M and N CTX-M-1 strains, plasmids also belonged to incompatibility group I1/Iγ and shared the same γ restriction pattern (Table 4). Some minor polymorphisms were observed between plasmids exhibiting β and γ patterns, suggesting microevolution in different bacterial hosts (data not shown).

DISCUSSION

Our first result derived from the long-term monitoring of antibiotic usage and ESBL-E carriage rates in the Trois-Sauts village from 2001 to 2010. There was a trend toward a correlation between the two parameters, albeit not statistically significant. This may be because only three observation points were available and because the sizes of the cohorts were relatively small. In addition, after adjustment for multiple testing, no individual risk factor, including antibiotic exposure within the year preceding each sampling, was associated with an increased individual ESBL-E carriage rate. Thus, altogether our results may suggest that it is the overall

TABLE 4 Phenotypic analysis and β-lactamase molecular identification of the *E. coli* strains and their transformants

Year of sampling	Strain (no. of isolates)[a]	Isolate no.	ESBL	Phylogenetic group/subgroup[b]	Virulence gene(s)[c]	Presence of TEM-1	Coresistance to antibiotics other than β-lactams[d]	Transfer in *E. coli* recipients[e]	Resistance trait(s) cotransfered with ESBL gene[d]	Plasmid replicon type(s) in recipients	Plasmid restriction profile
2006	A (1)	S028Ha	CTX-M-8	A_0	*aer*, *iroN*, *traT*, *hra*, *kpsE*	−	SSS, TMP	T	None	NT[f]	ζ
	B (2)	S041Ha	CTX-M-2	B1	*iroN*, *papC*, *traT*, *hra*	+	K, NA, CIP, TE, SSS, TMP	No	NP[g]	NT	NP
	C (7)	S055Ha	CTX-M-2	A_1	*iroN*, *traT*	−	SSS, TMP, TE	C	TE	FIB, I1/Iγ	α
	D (1)	S058Ha	SHV-2	A_0	*aer*, *iroN*, *traT*	−	None	C	None	I1/Iγ	NP
	E (1#)	S122Ha	CTX-M-2	B1	*ompT*, *traT*	+	K, SSS, TMP, TE	C	K, SSS, TE	HI1	NP
	F (1#)	S122Hb	CTX-M-2	D_2	*aer*, *iroN*, *traT*	+	NA, SSS, TMP	T	None	NT	NP
	G (1)	S141Ha	SHV-2	D_2	*ompT*, *hra*	−	G, K, T, Net, TE, SSS	C	G, K, T, Net	I1/Iγ	NP
2010	K (1*)	HE001.1	CTX-M-8	A_0	*ompT*, *traT*	−	K, TE, SSS	T	SSS	I1/Iγ	β
	L (1*)	HE001.2	CTX-M-2	A_1	*aer*, *kpsE*, *traT*, *fyuA*, ***afaD***	−	TE, SSS, TMP	C	TE, SSS, TMP	FIB, HI2	NP
	M (3†)	HE054	CTX-M-1	B1	*traT*, *hra*	−	NA, TE, SSS, TMP	C	SSS, TMP	I1/Iγ	γ
	N (3†)	HE055	CTX-M-1	B1	*aer*, *iroN*, *ompT*, *traT*	−	TE, SSS	C	TE, SSS	I1/Iγ	γ
	O (1)	HE071	CTX-M-2	B1	*aer*, *iroN*, *traT*	+	SSS, TMP	T	None	I1/Iγ	δ
	P (1)	HE090	CTX-M-8	D_2	*aer*, *fyuA*, ***aatA***, ***aaiC***	−	TE, SSS, TMP	T	None	NT	ε
	Q (1¶)	HE113.1	CTX-M-8	A_0	None	−	None	C	None	I1/Iγ	β
	R (1¶)	HE113.2	CTX-M-8	B1	*traT*, *fyuA*	−	None	T	None	I1/Iγ	β
	S (1¶)	HE113.3	CTX-M-8	A_1	None	−	None	C	None	I1/Iγ	β

[a] #, *, and ¶, the same volunteer was carrying two different strains; †, the strains were isolated from the same four volunteers.
[b] Determined as in Escobar-Paramo et al. (28).
[c] Intraintestinal virulence genes are indicated in bold. Genes underlined are plasmid borne.
[d] SSS, sulfamethoxazole; TMP, trimethoprim; NA, nalidixic acid; TE, tetracycline; K, kanamycin; CIP, ciprofloxacin; G, gentamicin; T, tobramycin; Net, netilmicin.
[e] T, transfer by electroporation; C, transfer by mating.
[f] NT, not typed.
[g] NP, not performed.

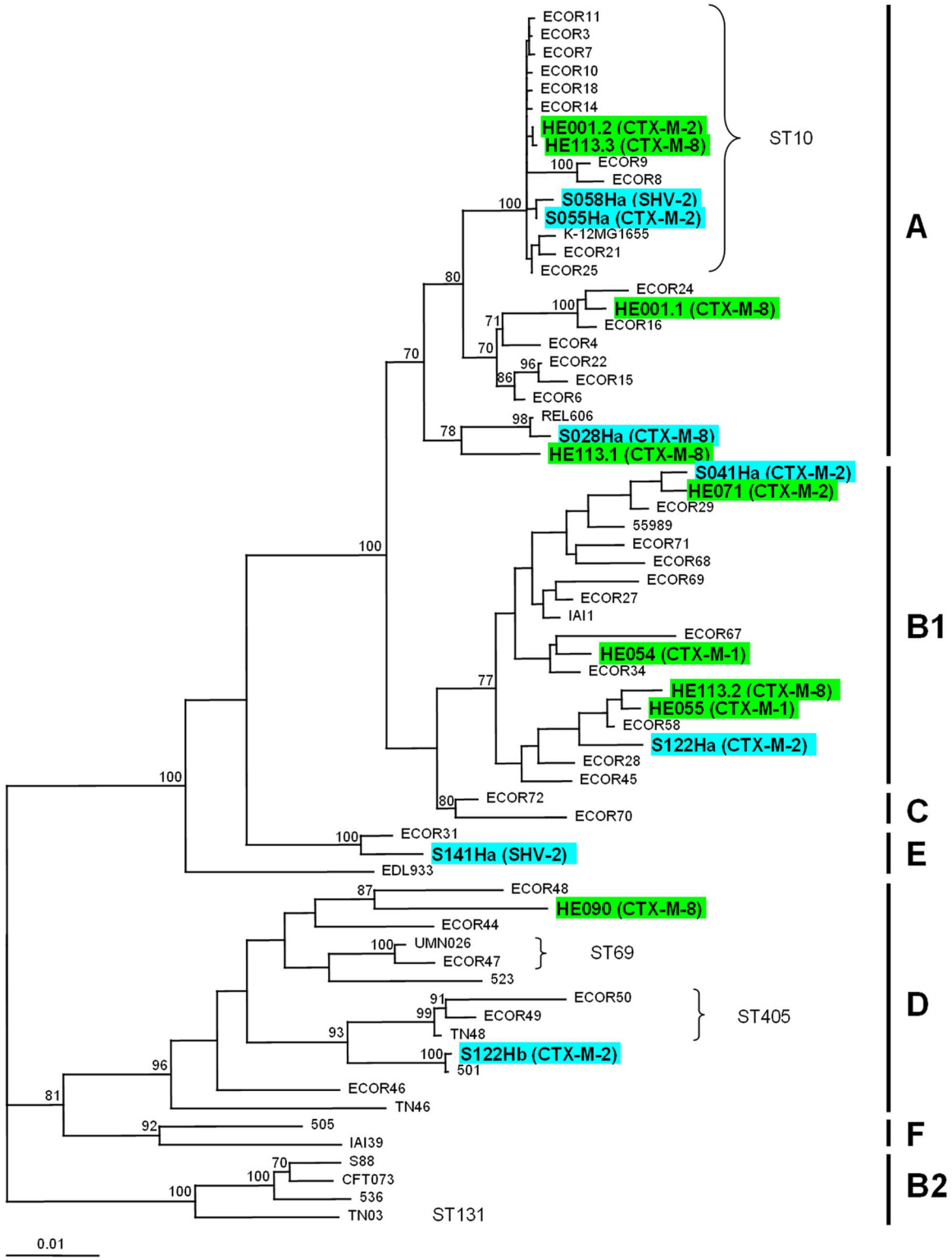

FIG 2 Phylogenetic tree of 67 *Escherichia coli* strains reconstructed from the concatenated sequences of six complete genes (*icd*, *pabB*, *polB*, *putP*, *trpA*, and *trpB*) corresponding to 6,023 bp using PHYML (24). The tree has been rooted on the B2 phylogroup as it is known to be basal in the *E. coli* phylogeny (25). Bootstrap values are indicated at the nodes when greater than 75% (500 replicates). The main phylogenetic groups of the *E. coli* species are indicated on the right of the figure. Seven and nine strains were isolated during the 2006 (blue) and 2010 (green) campaign samplings, respectively. The remaining strains were selected as representative of *E. coli* phylogenetic diversity (10). The TN48, UMN026, and TN03 strains are representative of multidrug-resistant spreading clones ST405, ST69, and ST131 according to the scheme of Wirth et al. (26), respectively. The ST10 encompassing the K-12 MG1655 strain is also indicated. Of note, the S141 Ha strain, typed as D_2 by the classic triplex PCR assay (8), belonged to the E phylogroup (27). β-Lactamase enzymes produced by the strains are indicated in parentheses.

exposure of a community to antibiotics which is a key for curbing ESBL-E carriage rates. This would be consistent with the results of a recent study performed in a small rural community in the United States, where the emergence of carriage of resistant *E. coli* was linked to intrafamilial transmission and not to individual exposure to antibiotics (15). Indeed, intrafamilial transmission of resistant fecal strains has been shown to occur in the absence of antibiotic pressure (16, 17). This is possibly what also occurred for some ESBL-E strains in Trois-Sauts.

The second result of our study was the observation that the dissemination of ESBL genes is more likely to be due to plasmid spread than bacterial strains. As observed in other household settings (18, 19), ESBL *E. coli* strains isolated in 2010 in Trois-Sauts were diverse. The two most prevalent *E. coli* clones were shared exclusively by three separate volunteers. In addition, no ESBL-E clone identified in 2006 was still present in 2010 in our cohort, suggesting limited persistence in the community over time. In contrast, ESBL plasmids sharing the same patterns were present not only among unrelated strains from one individual but also in strains isolated from different individuals. This suggested that once introduced in gut flora, plasmids spread among digestive *E. coli* strains, as demonstrated in gnotobiotic mice and in humans (20, 21). The presence of plasmids in commensal *E. coli* strains well adapted to their hosts may explain why CTX-M genes persist, even in the absence of any antibiotic selective pressure (22).

Both in 2006 and 2010, the plasmid determinants found in association with the $bla_{\text{CTX-M-2}}$ and $bla_{\text{CTX-M-8}}$ genes were very diverse. These are, indeed, the most prevalent CTX-M alleles in South America (23), suggesting, as observed in 2006 (6), that ESBL-E strains were continuously imported into the village from outside. The 2010 appearance of the $bla_{\text{CTX-M-1}}$ gene may also be the result of the importation of strains from outside the village. Although borne by two different clones identified in four volunteers, the $bla_{\text{CTX-M-1}}$ gene was found in a single plasmid pattern, which further suggests plasmid dissemination between commensal *E. coli* strains after importation. This is in accordance with what has been observed in other remote places with other resistance traits (17) and also with the increase in exchanges with the outside world that occurred in Trois-Sauts between 2006 and 2010.

To our knowledge, our long-term observation of ESBL-E carriage has never been conducted in a stable population. Our results support policies aimed at reducing antibiotic exposure. Plasmids appear to be primarily involved in the dynamics of dissemination of $bla_{\text{CTX-M}}$ genes in the community.

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